

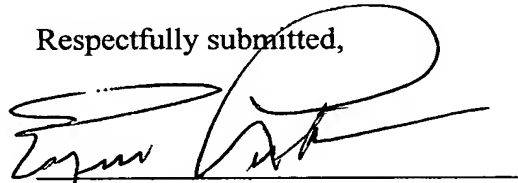
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REMARKS

Claims 7, 9, 16, 18, 22, 25, and 26 have been amended herein each for the first time to correct improper multiple dependencies therein. New claims 27 to 50 have been added. The clean version of these claims is attached in the Appendix hereto.

It is respectfully requested that the application should now proceed to prosecution.

Respectfully submitted,

A handwritten signature in dark ink, appearing to read 'Eugene Lieberstein', is written over a horizontal line.

Eugene Lieberstein
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Registration No. 24,645

Dated: July 3, 2003

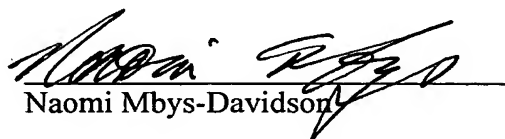
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Naomi Mbys-Davidson

APPENDIX

Clean Version of Claims 7, 9, 16, 18, 22, 25, and 26:

7. The method of claim 1, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

9. The method of claim 2, wherein the restriction enzyme is a class IIS restriction enzyme.

16. The method of claim 10, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

18. The method of claim 11, wherein the restriction enzyme is a class IIS restriction enzyme.

22. The method of claim 19, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

25. A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 1, into a target polynucleotide;

and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

26. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 1 into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

New Claims 27 to 50:

27. The method of claim 2, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

28. The method of claim 3, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

29. The method of claim 4, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

30. The method of claim 5, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

31. The method of claim 6, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

32. The method of claim 3, wherein the restriction enzyme is a class IIS restriction enzyme.

33. The method of claim 11, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins,

hormones, cytokines and plasma proteins.

34. The method of claim 12, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

35. The method of claim 13, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

36. The method of claim 14, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

37. The method of claim 15, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

38. The method of claim 12, wherein the restriction enzyme is a class IIS restriction enzyme.

39. The method of claim 20, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

40. The method of claim 21, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

41. A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 10, into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

42. A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 19, into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

43. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide

encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 10 into a target polynucleotide;
and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

44. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 19 into a target polynucleotide;
and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

45. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant

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polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by a method for evolving a polypeptide and a polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having additional nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

46. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by a method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut

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terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step a;

c) subjecting both cut termini of the target DNA obtained in Step b to self-ligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and

d) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

47. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having substitutive nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

48. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step a;

c) subjecting both cut termini of the target DNA obtained in Step b to self-ligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and

d) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

49. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut

terminus of the target DNA;

c) inserting a multiple of three substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having substitutive nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

50. A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by a method for evolving a polypeptide and a

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polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having additional nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a

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desired property and a polynucleotide encoding same as a target polynucleotide.